REMARKS

At the outset, Applicants acknowledge, with thanks, the withdrawal of all previous objections and rejections. The new grounds of rejection are addressed in the following.

Claims 18, 19, 21-35, 37-49, 62, 63, and 65-75 are pending. Claims 74 and 75 are withdrawn as being drawn to a non-elected invention.

Claims 18, 19, 21-35, and 37-49 are cancelled herein without prejudice. Upon entry of this amendment, claims 62, 63, and 65-73 will be under examination.

Claim objections:

Claims 18, 22-24, 34, 38-40, 62, 63 and 66-68 are objected to. The specific objections are addressed as follows below. The subject amendments do not narrow or broaden the scope of the claims.

The cancellation of claims 18, 22-24, 34 and 38-40 herein without prejudice renders the objections to those claims moot.

The Office Action suggests replacing the terms "said amplification regimen" in step (VII) of claim 62 with "said multiplex amplification regimen" to maintain consistency within the claim. Applicant has made the suggested amendment. The Office Action also suggests replacing the terms "said group of polymorphic sites" and "the set of target polymorphic sites" in claim 62 with "said group of known polymorphic sites" to maintain consistency within the claim. Also suggested is replacement of the phrase "distinctly sized amplification product" in step (a) of claim 62 with "plurality of distinctly sized amplification products." Applicant has made the suggested changes.

Applicant has corrected the typographical error in claim 63.

The Office Action suggests replacing the term "said amplification regimen" in claims 66-67 and the term "said regimen" in claim 69 with "said multiplex amplification regimen" to maintain consistency with claim 62. Applicant has made the suggested changes. Where the

amendments do not change the scope of the claims and were suggested by the Office Action, entry of the amendments is respectfully requested.

Rejection under 35 USC § 112, 2nd paragraph:

Claim 70 is rejected under 35 U.S.C. 112, second paragraph as being indefinite. The Office Action states that there is insufficient antecedent basis for the terms "said first and/or second tag sequences" recited in the claim. Applicant has amended the claim to recite "wherein said tag sequences each comprise 15 to 40 nucleotides." Reconsideration and withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. § 102:

Claims 18, 19, 21, 22, 26, 27, 34, 35, 37, 38, 42 and 43 are rejected under 35 U.S.C. § 102(e) as being anticipated by Chen et al. (U.S. 2003/0096277 A1). The cancellation of claims 18, 19, 21, 22, 26, 27, 34, 35, 37, 38, 42 and 43 without prejudice renders the rejection moot.

Rejections under 35 U.S.C. §103:

Claims 23-25 and 39-41 are rejected under 35 U.S.C. §103(a). The cancellation of claims 23-25 and 39-41 without prejudice renders the rejection moot.

Claims 28-33, 44-49, 62, 63, 65, 66 and 69-73 are rejected under 35 U.S.C. §103(a) as obvious over Chen et al. in view of Nolan et al. (U.S. 6,287,766). This is addressed in the following.

A) The cancellation of claims 28-33 and 44-49 without prejudice renders the rejection moot with respect to those claims.

B) With regard to claim 62 and its dependents, the proposed combination of Chen et al. and Nolan et al. does not provide the proposed benefit and fundamentally changes the nature of Chen et al.

The Office Action concludes that it would have been obvious from the teachings of Nolan et al. to incorporate an exonuclease digestion step following the initial primer extension reaction with the allele-specific primers, because "[a]n ordinary artisan would have realized from the teachings of Nolan that incorporation of an exonuclease digestion step following the initial primer extension reaction would have improved the methods of Chen by eliminating the possibility of mispriming events." This conclusion is incorrect for at least two reasons: i) mismatch extension, to the extent that it may occur in the method of Chen et al., only impacts the initial, allele-specific primer extension reaction, meaning that digesting the initial primer extension primers ***after*** the initial primer extension reaction will ***not*** prevent mismatch extension; and ii) Chen et al. teaches a different method of avoiding mismatch extension that is fully effective – adding the primer digestion step of Nolan et al. to the method of Chen et al. will not improve Chen et al. because it will not prevent mismatch extension in the allele-specific priming step, and replacing the mismatch avoiding step used by Chen et al. with the primer digestion both impermissibly changes the fundamental nature of the Chen et al. method *and* will not prevent mismatch extension. These issues and others are discussed in detail below.

i) **Mispriming and mismatch extension are different phenomena, and removal of unincorporated primer extension primers after the primer extension step will not avoid either.**

The Office Action states that the incorporation of an exonuclease digestion step as taught by Nolan et al. "would have improved the methods of Chen by eliminating the possibility of *mispriming* events." From this statement it appears the Office Action may be misinterpreting the Chen et al. reference and the "mismatch extension" to which it refers. Nolan et al. says nothing at all about mispriming and nothing at all about mismatch extension, which are two different phenomena. Central to the Chen et al. reference is the avoidance of *mismatch extension* – that is, inappropriate read-through of a mismatched 3' nucleotide on an allele-discriminating primer. Because allele discrimination according to the Chen et al. method depends upon the inability of a polymerase to extend a primer unless the 3' terminal nucleotide of the primer is base-paired with the template (e.g., at a SNP site), polymerase read-through of a mismatched

3' nucleotide will render the method of Chen et al. ineffective. Mismatch extension is **not** mispriming. Rather, mispriming is promiscuous or non-specific priming – where, for example, under given conditions, a primer initiates polymerization from other than its intended target. See, for example, Chou et al., Nucleic Acids Research 20: 1717 (1992) (**Exhibit A**), which teaches that mispriming, which leads to primer dimers and other artifacts, can be avoided, for example, using "Hot Start" PCR. Applicant submits that removal of primer extension primers before PCR will not necessarily change the rate of mispriming, and would not, as stated by the Office Action, be effective for "eliminating the possibility of mispriming events."

Similarly, removal of the primer extension primers by nuclease digestion will *not* eliminate mismatch extension. Any mismatch extension occurring in ***either*** the methods taught by Chen et al. *or the claimed method* **necessarily occurs in the allele-discriminating polymerization** – that is, in the primary PCR (in the context of Chen et al.'s method) or in the primer extension (in the context of the claimed method; see, e.g., claim 62). That is where mismatch extension would be detrimental to the ultimate detection of the specific SNP allele, as that is the only step that relies upon the ability of a polymerase to faithfully extend only those primers that are annealed at their 3' nucleotide (and, conversely, upon the inability of the polymerase to extend from a mismatched 3' terminal nucleotide). Removal of the primers *after* that first, allele-discriminating polymerization step will not avoid or eliminate the possibility of mismatch extension, and particularly not mismatch extension that could be detrimental to the detection of the SNP, because mismatch extension could have already occurred in the primer extension or primary PCR step. Removing the unincorporated primers after the mismatched primers have been extended will not fix or eliminate the problem. As such the proposed motivation to add a primer digestion step to the method of Chen et al. fails of its own accord. Applicant notes that the primer degradation of claim 62, step (V) is performed for reasons other than avoiding mispriming or mismatch extension.

In view of the above, Applicant submits that removing the primary PCR primers taught by Chen et al., using, for example, nuclease digestion as taught by Nolan et al., will not eliminate the possibility of mispriming events, nor will it eliminate the possibility of mismatch extension. As such, neither would provide the proposed motivation to modify the methods of Chen et al. to arrive at the claimed invention.

ii) Chen et al. teaches its own method of avoiding mismatch extension that is fully effective – there is no reason to "improve" Chen et al.'s method of avoiding mismatch extension even if the digestion could do so.

The Chen et al. reference demonstrates, e.g., in paragraphs [0088] and [0089], that the extension of mismatched primers is not a problem in the methods they teach. These paragraphs are reproduced below for convenience:

> [0088] In this study M13 reverse primer was used as the connecting element and two bases (GG and CC) as the coupling elements. The use of two bases for the coupling elements were based on previous reports (21, 22) that *two consecutive mismatch bases were sufficient to block the extension of DNA polymerases.* In the practice of the present invention, the coupling elements serve two goals: i) to connect the primary and secondary reaction allele-specifically, and ii) to increase the overall allele discrimination. *It is thus important to demonstrate that no mismatched extension occurs at the secondary reaction.*
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> [0089] *In order to do so, the following experiments were conducted*: Several DNA samples with known genotypes for marker SC.sub.--31, either homozygous allele 1 (G/G) or homozygous allele 2 (C/C), were chosen to for the experiments. In the primary reactions, only one of the two allele specific primers that matched the known genotypes of the DNA samples was used so that only one allele was amplified in the reactions. *In the secondary reactions, both allele specific primers were used so the occurrence of mismatched extension could be detected. In these experiments, if the two base mismatches (the coupling element) between the primary and secondary primers were sufficient to block the extension of the mismatched primer, we would expect that only the matched primers would produce extension products.* If products from both matched and mismatched primers were seen, the ratio of the two products would reflect the difference of efficiency between the matched and mismatched primers, or the blocking efficiency of the two base coupling elements. The results from the experiments are shown in FIG. 3. As can be seen, when the genotype of the sample was G/G homozygous only the corresponding secondary primer, which was labeled with BFL, produced a product (FIG. 3, panel A and B, peak indicated by arrow). The

> secondary primer corresponding to the C allele, which was labeled with BTAMR, did not have any detectable products. When the genotype of the sample is C/C homozygous the patterns reversed, only the BTMR-labeled secondary primer produces a peak (FIG. 3, panel C and D, peak indicated by arrow). *These experiments demonstrate that two consecutive mismatch bases are sufficient to block the extension of the mismatched secondary primers and do not produce unintended extension products detectable by DNA sequencers.* All products observed, therefore, were directly linked to the primary reactions. The results showed that the coupling of the two AS-PCRs was highly allele-specific. (Emphases added)

Specifically, the Chen et al. reference teaches the successful use of a dinucleotide coupling element to block the extension of mismatched primers. Thus, the nuclease digestion step proposed in the Office Action would not provide an improvement over the methods taught by Chen et al. because mismatch primer extension was specifically shown by Chen et al. not to be a problem in their methods as taught.

iii) Substituting a primer digestion step for Chen et al.'s method of avoiding mismatch extension not only won't work, but would fundamentally change the principle of operation of the Chen et al. method.

Further, Applicant submits that the use of a "coupling element" to avoid mismatch extension is central to the methods taught by Chen et al. See, e.g., paragraphs [0041] – [0043] and Example 1 (paragraphs [0087] to [0089]). Example 1, titled "Coupling the two levels of AS-PCRs" states that "[t]he coupling of primary AS-PCR with a secondary AS-PCR serves two purposes: one is to limit the undesired mismatch extension of primary AS-PCR, the other is to reduce the cost of genotyping" (paragraph [0087]). While Applicant in no way admits that primary primer digestion would eliminate mismatch extension (or, for that matter, mispriming), the use of digestion as an alternative method of reducing mismatch extension would necessarily change the operating principle of the primary Chen et al. reference, which relies on the design of the coupling element to avoid mismatch extension. It is well settled that if the proposed combination of the prior art would change the principle of operation of the prior art being modified, then the teachings of the references are not sufficient to render

the claims obvious. See *MPEP* § 2143.01 (citing *In re Ratti*, 123 USPQ 349 (CCPA 1959)).

iv) Because Chen et al. specifically teaches that the primary PCR and secondary PCR can be performed in the same reaction mixture, the presence of the primary PCR primers during the secondary PCR is not detrimental.

Chen et al. teaches that the primary and secondary PCR reactions in their described "AS-PCR^2" approach can be combined. That is, Chen et al. teaches that the presence of primary PCR primers during the secondary PCR amplification is not a problem. See, for example, Chen et al. paragraphs [0009] to [0012], reproduced below for convenience:

> [0008] In one embodiment, the invention provides a method of genotyping one or more loci in a DNA sample. The method includes the steps of
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> [0009] 1) ***combining*** *a sample containing*
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> [0010] i) single stranded DNA or double stranded DNA, ii) *at least one primary primer specific for one locus on one strand of DNA in the sample* (the primary primer has a first homologous portion which hybridizes to one strand of DNA and a non-homologous portion which does not hybridize to the one strand of DNA) ***and*** *at least one secondary primer* having a second homologous portion which includes the sequences of the non-homologous portion of said primary primer;
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> [0011] 2) conducting polymerase chain reaction (PCR); and
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> [0012] 3) identifying amplicons of the PCR which include the non-homologous portion. The step of identifying allows the genotype of the one or more loci to be established. (Emphases added)

As a further example, paragraph [0059] of the Chen et al. reference further teaches that the primary and secondary PCR reactions can be combined, where it states:

> *The primary and secondary reactions of the present invention can be performed separately or combined together.* When the two reactions are combined, the T_m for the primary and the secondary primers should be designed to be different. The T_m difference between the primary and secondary primers provides an opportunity to perform the two reactions at different temperature zones. For

> example, one can use a higher annealing temperature to amplify target DNA using the primary primers. When sufficient amount of products from the primary reaction are accumulated, one then lowers the annealing temperature for the secondary reaction. For example, one could use 70°C as the annealing temperature for the primary reaction and cycle 10 times, then the temperature could be lowered to 50°C for 30 more cycles. (Emphasis added)

Because Chen et al. teaches that the primary and secondary PCR reactions can be performed together, the presence of the primary primers can clearly be tolerated during the secondary amplification in the methods taught by Chen et al. As such, there would be no reason to substitute or to add the digestion of primary primers cited in the Nolan et al. reference to the methods taught by Chen et al. Thus, the proposed motivation to combine teachings of Nolan et al. and Chen et al. further fails.

In summary then, with regard to the rejection of claim 62 and its dependents over the combination of Chen et al. with Nolan et al., the claimed invention is not obvious over the combination because Chen et al. does not teach the degradation of non-annealed oligonucleotide primers as required by claim 62, step (V), and because the *addition* ***or*** *substitution* of a primer digestion step as said to be taught by Nolan et al. to the method taught by Chen et al. will not improve the method of Chen et al. by eliminating the possibility of mispriming or mismatch extension events as posited by the Office Action. Chen et al. teaches its own, fully effective method of avoiding mismatch extension, so there would be no reason to make the change proposed in the Office Action. Further, the teaching of Chen et al. that the primary amplification primers can remain present during the secondary PCR means that it would not have been obvious to add a primer digestion step to the method taught by Chen et al. to arrive at the invention of claim 62 as amended.

In view of the above, if the art were combined as proposed the supposed benefit would not occur, so there is no motivation to combine the cited art and the proposed combination is improper. Thus, the proposed combination cannot support a conclusion of obviousness with respect to claim 62, in which step (V) recites nuclease digestion of

non-annealed oligonucleotide primers, or with respect to claims dependent from claim 62. Applicant respectfully requests reconsideration and withdrawal of the obviousness rejection as applied to these claims.

Dependent claims 67 and 68 are rejected under 35 U.S.C. §103(a) as obvious over Chen et al. in view of Nolan et al. and further in view of Woolley et al.

The Office Action states that "[t]he combined teachings of Chen and Nolan result in the methods of claims 28-33, 44-49, 62, 63, 65, 66, and 69-73, as discussed above." Applicant has addressed the proposed combination of Chen et al. with Nolan et al. with respect to claim 62 and its dependents above. Woolley et al. does not remedy the defects in the combination of Chen et al. and Nolan et al. with respect to the invention as claimed in claim 62 and its dependents. As such, the proposed combination of Chen et al. in view of Nolan et al. and Woolley et al. cannot render the claimed invention obvious. Reconsideration and withdrawal of the rejection is respectfully requested.

In view of the above, all issues raised in the Office Action mailed September 16, 2009 have been addressed herein. Reconsideration of the claims is respectfully requested.

A Petition for Extension of Time, Notice of Appeal and authorization to charge the necessary fees to Deposit Account No. 50-0850 are filed herewith. Authorization is hereby given to charge any further fees necessary or to credit any overpayment to this deposit account, referencing Docket No. 046264-065331.

Respectfully submitted:

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